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Resting Spores of *Cosmarium bioculatum*, Breb.

J. A. NIEUWLAND.

The material that is the subject for the present article was collected between May 1 and 14, 1907, on the University grounds in a small rill along the branch of the Michigan Central Railroad, between Niles, Michigan and South Bend, Indiana. The locality is rich in more or less uncommon algal material and the larger portion of the valuable specimens collected in the last few years for the department of Botany, in the line of fresh water algae, was obtained from the immediate vicinity, that is within a radius of a half mile. Until very lately the surface water of the surrounding higher land collected in small pools along the railroad, and the rill from which this material was obtained had its origin in an old barrel that had formerly enclosed a spring. Its water flows along the tracks of the road into the St. Joseph River. Drainage systems have lately been put in and usefulness of the locality in the production of algal material is very much impaired. From July to November the streams and pools were completely dried up except after excessive rains and even then they were dried up in a few days. The small stream was sluggish in its flow at intervals thus affording places for the lodgement of the plankton in the weeds and grass. The plankton was so abundant that it could be collected by the gallon. About the 15th of May the desmid material contained mostly *Cosmarium bioculatum*, Breb., *Staurostrum rugulosum*, Breb., *Staurostrum polymorphum*, Breb. *Cosmarium botrytis*, Menegh., and *Spirogyra tenuissima* (Hass.) Kutz., with lateral conjugation only, *Zygnema insigne*, (Hass.) Kutz., in fruit, various forms of diatoms, *Vaucheria sessilis*, (Vauch.) DC., *Vaucheria geminata*, (Vauch.) DC., *Draparnaldia glomerata* (Vauch.) DC., *Palmella hyalina*, Breb., and *Chaetophora elegans*, Ag., also several forms of unicellular *Myxophyceae*. The desmid material was replaced by *Mougeotia scalaris*, Hass. so abundantly in the state of sexual reproduction that we considered it the best material we have ever found in that stage. The following algae were also present in the desmid material and all were abundant at different times between the beginning and the end of May: *Cosmarium intermedium*, a very small form of *Penium margaritaceum*, Breb. several species of monoecious and dioecious

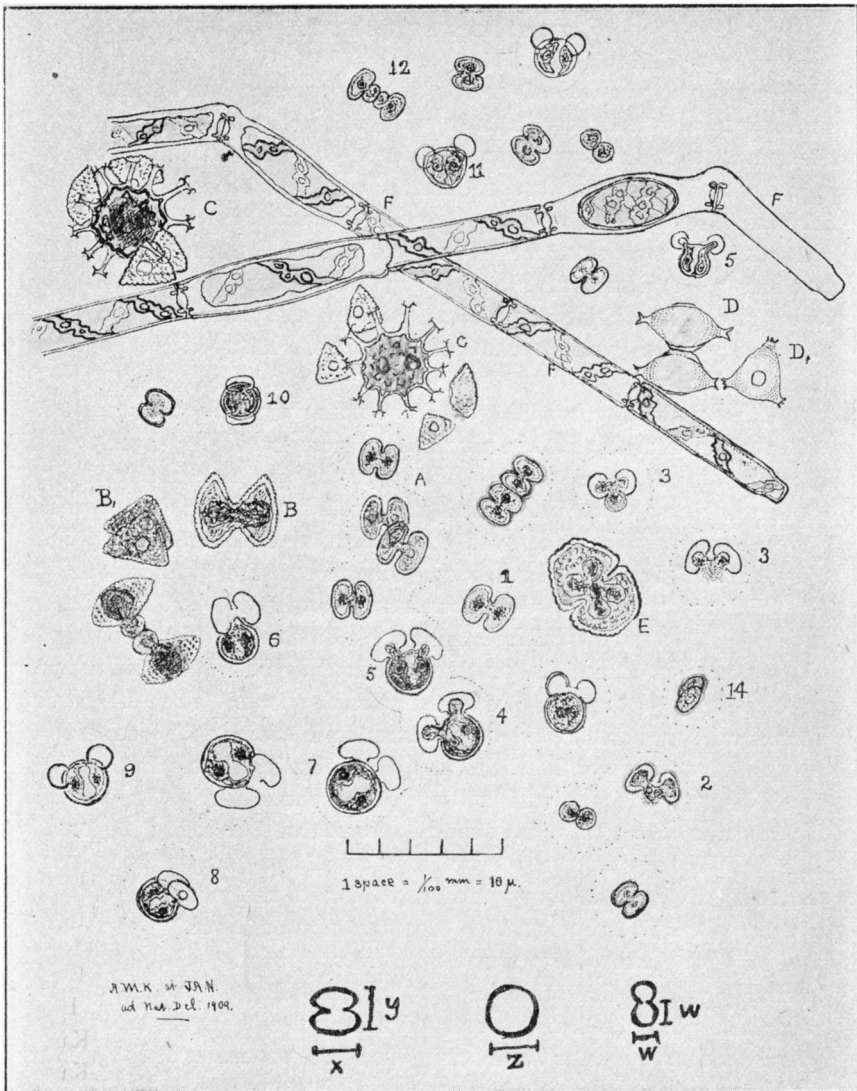


PLATE I.

ASEXUAL RESTING SPORES OF COSMARIUM BIOCVLATUM, BREB.

Figures 1-11. Stages of formation of resting spore.

1. Vegetative stage. 2-3. Separation of the semicells with formation of vesicle. 4-6. Later stages of formation of spore from the vesicle. 7-11. Fully formed spore with empty semicells in various position in the surrounding mucilage. 12. Cosmarium bioculatum, vegetative divisions. 14. End view of vegetative stage. A. Same as 1, front view. B. Staurastrum rugulosum, Breb. B. End view of same. C. Formation of zygospore of same. D. Staurastrum polymorphum, Breb. Side and end view of vegetative stage. E. Cosmarium botrytis, Menegh. F. Spirogyra tenuissima, (Hass.) Kütz.

W. X. Y. Z. Measurements of Cosmarium bioculatum.

Side View, W. = 6.75μ

Front View { X. = 13.50μ — 14.62μ .

{ Y. = 13.50μ — 15.75μ .

Zygospore, Z. = 13.5μ — 24μ .

(Usual size, 18μ diam.)

Oedogonia, a very small form of monoecious *Bulbochaete*, all more or less abundantly in fruit. Not more than a hundred yards from this spot in a pool was found *Mougeotia mirabilis* Al. Br. in all stages of sexual reproduction by lateral conjugation and in such large quantities that it could be raked out of the water in masses of two or three pounds at a time. Some miles away another pool was found containing *Cosmarium botrytis* rather abundantly in conjugation, and *Penium margaritaceum* not so commonly fruiting and a small amount of several species of vegetative *Closterium*.

The water containing the *Cosmarium bioculatum* was collected in vessels and the plankton concentrated by decantation of water, and taken to the laboratory where the material was studied. Some was fixed and the rest kept alive in aquaria. Several quarts were taken daily between the first and the 13th of May, after which date the specimens began to disappear gradually. Material transferred to the laboratory was invariably arrested in its growth after removal from its native habitat. The *Cosmarium bioculatum* continued to produce asexual spores but subsequent development of the full developed spores ceased. The full grown spores of the *Cosmarium bioculatum* and the zygospores of the other forms remained in the laboratory for a long time but neither could be brought to germination. After persisting in the aquaria for months alive they eventually disappeared, leaving no trace. The following year the stream and all the adjacent pools were carefully examined every few days for a reappearance of the plants. Only a few vegetative *Staurostrum* specimens were found and no *Cosmarium bioculatum* whatever. From the live material examined at the time of collection the following observations were recorded. The fixed and preserved material now in the laboratory jars is quite as good for the description of the processes as it contains all the various stages in the formation of the spores.

Staurostrum rugulosum, Breb. and *Staurostrum polymorphum*, Breb. were found, mostly in the latter stages of zygosporic reproduction the former so abundantly that the zygospores are more common in the material than the vegetative forms. Few early stages were found most of the zygospores having already fully developed spines with hooks.

Both vegetative and asexually reproducing stages of *Cosmarium bioculatum*, were extraordinarily abundant, though vegetative division of the cells was uncommon, and all the stages of the formation of the resting spore could be studied. These

various stages are shown in the plate in figs. 1-11. There was no need of hunting for the intermediate stages as nearly every particle of plankton contained them all, and often the field of a one-eighth microscopic objective with one inch ocular presented a complete series of stages of spore formation.

Following is a description of the process of the formation of the resting spore. Two semicells of the desmid, *Cosmarium bioculatum* separated from one another as the plants usually do in the formation of zygospores, only one individual, however, being engaged in the process. The protoplasm from each semicell emerged and formed a transparent globular vesicle. This vesicle gradually became invested with a thick cell wall on reaching full size. The two chromatophores with nuclear bodies each with a small body in it resembling a nucleolus on entering the vesicle took up positions at opposite pole of the vesicle only fusing their protoplasm along a thin median band, thus seeming at least in the early stages of the spore formation to maintain their individuality in the spore. The whole spore as also the vegetative stage of the desmid was invested with a thick mass of mucilaginous material. This fact was beautifully demonstrated in staining with diluted Delafield's Haematoxylin bringing up in alcohol counterstaining with Magdala Red and mounting in Venetian turpentine or balsam. The preparations thus made showed the cell wall of the desmid as also of the vesicle a rather deep purplish blue, the cell contents especially the chromatophores a bright red, and the mucilage coat pale lilac to purple. Thus it was easy to prove that one and only one desmid was engaged in the process of spore formation and therefore that it was not conjugation. Other preparations, using Magdala Red alone, showed the cell contents more clearly as the cell wall could be bleached to any degree of paleness or the stain quite removed by measured exposure to sunlight. Though thousands of the spores of *Cosmarium bioculatum* were examined under various conditions of staining and particularly in the study of the live material not a single case was found where more than one individual was noticed in the formation of the spore. The method of the formation of the spore is then a purely asexual one, unless the union of the contents of one semicell of an individual with those of its other half or of the other semicell can be considered a sexual process. The contents of each semicell of an individual can of course be supposed to become a gamete to the other half, and the resulting spore could in this sense be considered a zygospore by a degenerate form of conjugation.

A process has been described in which two individual desmids conjugate, each semicell of one with a corresponding semicell of another individual, the result being the formation of two zygospores from a single conjugation of only two desmids. In *Cosmarium bioculatum* only a single individual desmid was ever noticed to effect the formation of the spore. The latter was devoid of spines and resembled the ordinary zygospore reported for the individual.

Some attempts were made to obtain the subsequent stages of the development of resting spore in the laboratory and in the field. Though the material remained in the spore state for months it eventually disappeared and in the laboratory this was principally due to the ravages of minute water insects that could not be eliminated from the aquaria. In attempts to study field material no better results were obtained. Late in the summer the stream had dried up, not however, before all the desmid forms had disappeared. The following year, as already stated, practically no desmids were found and absolutely no *Cosmarium bioculatum*. Nothing but some ordinary forms of algae such as *Vaucheria sessilis* and *Vaucheria dichotoma*, (Lyngb.) Ag. some small forms of *Spirogyra*, *Zygnema insigne*, and vegetative *Mougeotia scalaris*.

A Biological Study of *Noctiluca miliaris*, Suriray.

A. M. KIRSCH.

Some months ago there was sent to the Zoological laboratory of the University of Notre Dame, a vial containing what proved to be *Noctiluca*, collected on the Oregon shore of the Pacific Ocean, and with the specimen a request was made what it might be. *Noctiluca* is a comparatively well known animal in zoological literature, and Dr. O. Bütschli in his work on the Protozoa, which forms the first volume of Bronn's "Klassen und Ordnungen" mentions no less than forty-one authors that speak either directly or indirectly of this highly interesting flagellate protozoan. Our experience above referred to illustrates that many workers in Zoology need more than the classical literature to identify the many curious animals they now and then meet with. It is not the primary purpose of the Midland Naturalist to publish exhaustive memoirs or treatises for specialists in Biology, but rather to give